Interaction of Impulsive Pressures of Cavitation Bubbles with Cell Membranes during Sonoporation

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Interaction of Impulsive Pressures of Cavitation Bubbles with Cell Membranes during Sonoporation

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Abstract. Ultrasound contrast agents (UCAs), are capable of enhancing non-invasive cytoplasmic molecular delivery in the presence of ultrasound. Collapse of UCAs may generate nano-scale cavitation bubbles, resulting in the transient permeabilization of the cell membrane. In the present study, we investigated the interaction of a cavitation bubble-induced shock wave with a cell membrane using acoustic theory and molecular dynamics (MD) simulation. From the theory, we obtained the shock wave propagation distance from the center of a cavitation bubble that would induce membrane damage. The MD simulation determined the relationship between the uptake of water molecules into the lipid bilayer and the shock wave. The interaction of the shock wave induced a structural change of the bilayer and subsequently increased the fluidity of each molecule. These changes in the bilayer due to shock waves may be an important factor in the use of UCAs to produce the transient membrane permeability during sonoporation.

Keywords: Shock wave, Cytoplasm, Molecular delivery, Ultrasound
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INTRODUCTION

Non-invasive, tissue specific molecular delivery is crucial for the efficacy and reduced side effects of a wide range of treatments. Ultrasound contrast agents (UCAs), which are encapsulated gas bubbles with a diameter < 10μm, have been developed for a non-invasive physical method to deliver drugs and genes into targets sites using ultrasound. However, this efficacy is relatively low, and the mechanism of gene transfer as well as the methodology has to be elucidated and optimized.

Although nano-scale cavitation bubbles created by collapse of UCAs are believed to be a major cause for molecular delivery in this method, the detailed mechanism has not been understood. In general, cavitation bubbles generate impulsive pressures such as liquid jets and shock waves. The direction and intensity of the liquid jet depends on the dynamic response of the surrounding materials[1, 2]. The generation and rapid expansion of cavitation bubbles drive the surrounding liquid outwards, resulting in the pressure waves near the bubble walls. The pressure waves propagate outwards as

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shock waves. The shock waves interact with the surrounding cell membranes, leading to transient membrane permeability, followed by entry of exogenous molecules. Kodama et al.[3] reported that the shock wave impulse (defined as the integral of pressure with duration) is an important factor governing the temporary permeability increase in cell membranes.

In this study, we first carried out numerical analysis of a free spherical cavitation bubble motion that is generated by collapse of UCAs in the field of ultrasound. The interaction of a shock wave with a cell membrane was investigated. Next we conducted molecular dynamics (MD) simulation to investigate the interaction of the shock wave with the cell membrane on the molecular level.

THEORY AND MOLECULAR DYNAMICS SIMULATION

Bubble dynamics

Assume that cavitation bubbles are generated from cavitation nuclei, proposed by the Harvey model[4]. This model is based on an assumption that gas is trapped within a crevice and a cavitation bubble is generated from the crevice due to the decrease in pressure. Suppose that the gas of UCAs, C_3F_8, is trapped within crevices of the debris produced by collapse of UCAs and cavitation bubbles are generated from the crevices, and behave as shown in Eq.(1). The pressure at the bubble wall is given by Eq.(2).

The radial motion of a bubble with a radius R in compressible and Newtonian liquid, a Keller-Miksis model [5], is expressed as

$$R\ddot{R}\left(1-\frac{1}{C_L}\dot{R}\right)+\frac{3}{2}\dot{R}^2\left(1-\frac{1}{3C_L}\dot{R}\right)=\left(1+\frac{\dot{R}}{C_L}\right)\frac{1}{\rho_L}\left[P_{r=R}(t)-P_C\left(t+\frac{R}{C_L}\right)-P_\infty\right]$$
$$+\frac{R}{\rho_L C_L}\frac{dP_{r=R}(t)}{dt} \tag{1}$$

The pressure, $P_{r=R}$, at the bubble surface is given as

$$P_{r=R}(t)=\left(P_\infty+\frac{2\sigma_L}{R_0}\right)\left(\frac{R_0}{R}\right)^{3\gamma}-\frac{2\sigma_L}{R}-\frac{4\mu_L}{R}\dot{R} \tag{2}$$

The oscillation pressure term P_C is given as

$$P_C(t)=|P_A|\sin\omega t \tag{3}$$

where, C_L is the sound velocity in liquid (1497.3 m/s), P_∞ atmospheric pressure (101.3kPa), R_0 initial bubble radius, γ adiabatic exponent of a gas, μ_L liquid shear viscosity (0.890mPa·s), P_A peak positive pressure measured in the experiments, ω circular frequency. Consider that a spherical shock wave is generated at the bubble rebound. The shock wave interacts with surrounding cells, resulting in the cell membrane disruption. The radius from the center of the bubble, r_C, for generating the disruption of the cell membrane is expressed as [6]

$$r_C \approx \frac{P_{\max}R_{\min}}{\varepsilon_C \rho_L c_L^2} \tag{4}$$

where $P_{\max}$ was the maximum pressure at the rebound when the bubble obtained the minimum radius $R_{\min}$, and ε_C was the static critical strain necessary to irreversibly disrupt the membrane. ε_C was estimated to be 0.02-0.03 for red blood cells [7].

The impulse, I_{rc} of the shock wave at $r = r_C$ is given as

$$I_{r_C} = \frac{\rho_L C_L E_S}{2\pi r_C R_{\min} P_{\max}} \tag{5}$$

The calculation was conducted for 50 μs using the 4th order Runge-Kutta method.

Molecular dynamics simulation

Following the method developed by Koshiyama et al.[8], the interaction of a single lipid bilayer with a shock wave was calculated. The lipid bilayer was designed as a 32 dipalmitoyl phosphatidylcholine (DPPC) lipid bilayer, placed between two 1200 water layers in the rectangular calculation box. This box was a cubic whose longitudinal axis was set to the *z*-axis perpendicular to the *xy*-plane. A single shock wave was characterized by an impulse, I_p, that was expressed with a velocity V_2, determined by the change in the momentum. The V_2 was expressed as

$$V_2 = \frac{I_p}{M} A \tag{6}$$

where M (kg) was the mass of water molecules in the water layer, A (m^2) was the area of the *xy*-plane in the calculation box, and I_p was varied from 1.6 to 16 mPa·s. The impulse was applied downwards to the part of the upper water layer. The simulation was terminated when the wave generated by the impulse reached to the bottom surface of the calculation box. The simulation was performed using the AMBER 7 set of programs.

EXPERIMENTS

We used human embryonic kidney cells (293T) and cultured in RPMI 1640 containing 10% fetal calf serum and 1% penicillin-streptomycin in 250-mL culture flasks in a cell culture incubator. The luciferase reporter vector pGL3–Control and pCMVβ vector were obtained from Promega and Clontech, respectively. Two types of UCAs were used; Optison™ (Amersham Health Plc, Norway) and lipid bubbles. The inside gas of both bubbles was C_3F_8. The zeta potential of the bubbles was measured by a Zata Sizer ZS (Malvern Instruments, UK) in phosphate-buffered saline (PBS) without Ca^{2+} and Mg^{2+} (PBS, 1×). The bubble size was determined by a laser diffraction particle size analyzer (SALD-2000, Shimadzu Co., Japan). We used 945 kHz ultrasound that was generated by a submersible piezo ceramic transducer with 12mm diameter (Fuji Ceramics Co., Japan) in a test chamber filled with distilled water. The 48-well cell culture plates were located just above the ultrasound probe and cells were transfected in the wells.

RESULTS AND DISCUSSION

The medium containing of UCAs was initially white emulsified medium. After ultrasound exposure, the medium became transparent within a second, and white flowing debris was observed in the medium. As the height of the medium decreased,

the intensity of capillary waves at the medium surface increased, and subsequent atomized liquid particles started to fly from the surface. This rapid UCA collapse, significant instability of the free surface, and resultant atomized particles, indicates collateral evidence of the existence of cavitation bubbles in the medium. Suppose that the inside gas of UCAs, C_3F_8, is trapped within crevices of the debris produced by collapse of UCAs and cavitation bubbles are generated from the crevices, and behave as shown in Eq.(1).The pressure at the bubble wall is given by Eq.(2). Figure 1A shows that the periodic cavitation bubble motion in the presence of ultrasound. The impulsive pressure is generated at the bubble rebound, resulting in a spherical shock wave propagating outwards (Fig.1B).

Now we estimate the damage potential radius, r_C, by a single shock wave from the center of a cavitation bubble, given as Eq.(4). Figure 1C shows the relationship between the potential radius for causing the membrane damage by the shock wave and bubble expansion for varying ultrasound pressures. The results suggest that the cell membrane will be damaged by the interaction of the shock wave when cells are located at more than 5μm away from the center of the bubble.

Next we investigated the molecular structural change of the lipid bilayer with the shock wave on the molecular level. Figure 2A shows the structural change of the lipid bilayer obtained by MD simulation. The shock wave impulse was applied downwards. Water molecules were delivered into the hydrophobic region as the lipid bilayer was compressed downwards. The trend of the uptake of molecules was qualitatively consistent with the experimental data reported by Kodama et al.[3].

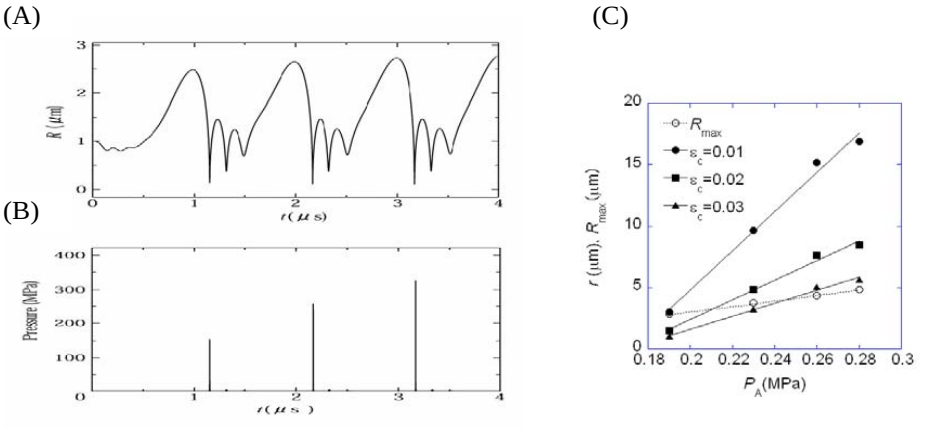

Figure 1. (A) Periodic cavitation bubble motion in the presence of ultrasound. (B) Impulsive pressure generated at the bubble rebound, resulting in a spherical shock wave propagating outwards. (C) Potential radius of inducing cell damage by a shock wave from the center of a cavitation bubble, and the maximum bubble expansion radius, R_{max}. Ultrasound pressure, P_A was varied from 190 kPa to 280 kPa. ε_C is the area strain of a cell membrane exposed to the shock wave, where R_0 = 1μm. Numerical data was obtained using the 4th orde Runge-Kutta method.

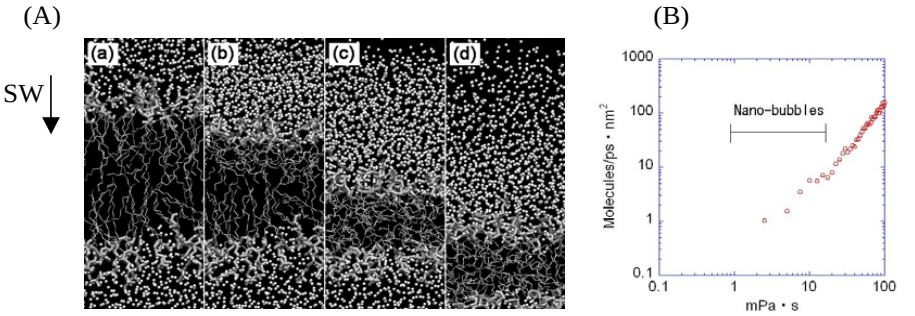

Figure 2. (A) Structural change in the lipid bilayer with a shock wave impulse. I_p= 40 mPa·s. SW: shock wave. (a) Equilibrium state, (b) 0.23 ps, (c) 0.46 ps, (d) 0.69 ps. (B) Normalized number of delivered water molecules into the bilayer per mm^2 and per ps. The solid bar indicates the impulse range created by a cavitation bubble with a radius of 1μm, obtained by Eq.(5), where the pressure P_A was varied from 190 kPa to 280 kPa.

Figure 2B shows the relation between the uptake of water molecules into the bilayer and the shock waves impulse. The number of water molecules increases with increasing shock wave impulse. The solid bar indicates the range of the shock wave impulse created by a cavitation bubble with a radius of 1μm. From the theory and MD simulation, it was found that the shock waves from cavitation bubbles, created by collapse of UCAs, were a dominant factor of molecular delivery into cells during sonoporation.

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REFERENCES

[1] T. Kodama and Y. Tomita, Applied Physics B-Lasers and Optics **70**, 139-149 (2000).
[2] Y. Tomita and T. Kodama, Journal of Applied Physics **94**, 2809 (2003).
[3] T. Kodama, M. R. Hamblin, and A. G. Doukas, Biophysical Journal **79**, 1821-1832 (2000).
[4] E. N. Harvey, D. K. Barnes, W. D. McElroy, et al., J Cell Comp Physiol **24**, 1-22 (1944).
[5] J. B. Keller and M. Miksis, J. Acoust. Soc. Am. **68**, 628-633 (1980).
[6] J. Sundaram, B. R. Mellein, and S. Mitragotri, Biophysical Journal **84**, 3087-3101 (2003).
[7] E. A. Evans, R. Waugh, and L. Melnik, Biophys J **16**, 585-595 (1976).
[8] K. Koshiyama, T. Kodama, T. Yano, et al., in *Fourth International Symposium on Advanced Fluid Information and the First International Symposium on Transdisciplinary Fluid Integration*, edited by T. Ikohagi, Sendai, 2004, p. 36-37.